Analysis of T Lymphocyte Subpopulation Change Defined by Monoclonal Antibodies Immediately after Radiotherapy

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We studied the T lymphocyte and its subpopulation percentage change in 40 patients immediately after the radiation therapy. Study population consisted of 12 patients treated at the site of head and neck region, 14 patients treated at the site of thoracic region, and 14 patients treated at the site of pelvic region. Twenty two patients received radiotherapy as radical modality, and remaining 18 patients received radiotherapy as postoperative modality.

Immediately after radiotherapy, total T lymphocyte (T1) percentage was decreased from 56.4% to 55.2%, helper T cell (T4) percentage was decreased from 36.4% to 34.1%, but suppressor T lymphocyte (T8) percentage was increased from 23.5% to 25.4%. As a result, T4/T8 ratio was decreased from 1.57 to 1.39. This study suggested that immediate change after radiotherapy of the T lymphocyte and its subpopulation percentage was not related to the treatment volume and the degree of helper T cell decrement was not pronounced by the radiation dose increment. Long-term follow-up study in larger scale is needed to determine long term changing pattern in T lymphocyte subpopulation and its relationship to the prognosis of patients.

Key Words: Radiation therapy, T lymphocyte subpopulation, Monoclonal antibodies

INTRODUCTION

T lymphocyte is postulated to play an important role as an effector cell responsible for immunologic surveillence resulting in elimination of tumor cells from the host. Numerous reports described the immune-specific T cell with antitumor reactivity and these findings prompted further studies on host immune defense in cancer patients. The studies of T cell functions in relation to patient susceptability for cancer reported conflicting data in immune responses against the cancer growth[1,2]. The lymphocytopenic effect of irradiation has been recognized for many years.

The various immunologic functions of lymphocytes measured in vitro assays are affected to different extents by radiation[3,4]. T-lymphocyte subpopulations showed varied radiosensitivity from extreme radiosensitivity of the precursor cell to radioresistance of effector cells and suppressor cells were particularly radiosensitive[5]. Immune surveillence theory causes some concern because of the detrimental effect of radiation on immune system, but it is not yet clear whether the lymphocytopenic effect of radiotherapy influences adversely the prognosis of irradiated patients. Many conflicting articles are co-existing[6~12]. In addition, the published data of different studies on the effect of radiation therapy on the T cell subpopulation are not consistent[3,6,11,13~16]. We have performed this study to confirm the immediate effect of irradiation on the T cell subset distribution and to establish the base line to evaluate long term changing pattern of T cell subset as a prognostic indicator in irradiated patients.

MATERIALS AND METHODS

1. Patients

Study population consist of 40 patients who were treated at the Department of Therapeutic Radiology, Gyeongsang National University Hospital from June 1991 to December 1991.

Patients treated with curative aim were entered into this study. Characteristics of patients is shown in Table 1. Eighteen of 40 patients were male and 22 patients were female.

Age ranged from 29 years to 78 years and median age was 57 years. Twelve patients received radiotherapy in the region of head and neck, 14 patients in the region of thorax and 14 patients in the region of pelvic area. Thorax treated group consisted of 5 patients with breast cancer, 4 patients with lung cancer, 4 patients with esophageal cancer, and 1 patient with thymoma. Pelvis

Table 1. Characteristics of Study Population

Characteristics	Numbers
Total entered	40
Sex	
male	18
female	22
Range of age in years	29~78
(median age)	57
Treatment site	
head and neck	12
thorax	14
pelvis	14
Aim of radiotherapy	
primary radical	22
postoperative	18

treated group consisted of 11 patients with uterine cervical cancer, 3 patients with rectal cancer. Site of cancer was variable in head and neck treated group. Mean treatment volume was 976 cm^3 in head and neck treatment group, 1,969 cm^3 in thorax treatment group, and 3,528 cm^3 in pelvis treatment group.

Twenty two patients received radiotherapy as radical modality, and 18 patients received as postoperative adjuvant modality. Radiotherapy dose ranged 4,500 cGy to 5,000 cGy in adjuvant treatment group and 5,500 cGy to 7,020 cGy in radical treatment group.

2. Determination of Lymphocyte Subpopulation

The blood was sampled into heparinized tube 1 day before initiation of radiotherapy and immediately after the completion of radiotherapy. The heparinized blood was diluted with equal volume of Hanks balaced salt solution (HBSS) and centrifuged with a cushion of Ficoll-Hypaque gradient at 600 × g for 30 minutes. The mononuclear cell band was harvested and washed twice in HBSS. Mononuclear cell smears prepared on glass slides were air-dried and either stained on the day of preparation or stored at −20℃ until staining. Monoclonal primary antibodies were T1 (mouse antihuman T cell, DAKO Corp.), T4 (mouse antihuman T helper cell, DAKO Corp.), T8 (mouse antihuman T suppressor cell, DAKO Corp.). Secondary antibody was antimouse immunoglobulin (Sigma chemical Corp.) We used APAAP complex which is monoclonal anti-mouse alkaline phosphatase antibody to enhance the detectability of surface antigen. Each reagent was diluted with Tris buffered Saline (TBS) and applied for 30~60 minutes with intervening washings in TBS. After incubation, the smears were washed once again and incubated with the naphthol AS-MX sodium salt/fast red TR salt. The red stained cells were scored as positive[17,18]. Results are given as mean value with standard error. Statistical analysis was done by paired t test.

Table 2. T lymphocyte Subpopulation Change Immediately after RT* (n=40)

	Before RT	Immed. after RT	t-value	significance
T1(%)	56.5±1.75	55.4±1.68	5.67	$p<0.01$
T4(%)	36.5±1.74	34.1±1.37	6.50	$p<0.01$
T8(%)	23.6±2.70	25.4±3.57	4.09	$p<0.01$
T4/T8	1.53±0.22	1.37±0.19	9.24	$p<0.01$

*RT: Radiotherapy

Table 3. T lymphocyte Change in Head and Neck Treated Group (n=12)

	Before RT	Immed. after RT	t-value	significance
T1(%)	56.5±1.84	54.9±1.39	9.21	$p<0.01$
T4(%)	36.4±1.52	34.1±1.02	4.45	$p<0.01$
T8(%)	23.5±3.26	24.9±3.04	1.91	NS*
T4/T8	1.57±0.22	1.39±0.16	2.47	$p<0.05$

*not significant

RESULTS

Immediately after radiotherapy, the average percentage of total T lymphocyte (T1) was changed from 56.4% to 55.2%. Helper T cell (T4) percentage was decreased from 36.4% to 34.1%. By contrast, percentage of suppressor T cell (T8) increased from 23.5% to 25.4%. This resulted in decline of T4/T8 ratio from 1.53 to 1.37 (Table 2). T cell subset distribution change was not different significantly among 3 population according to treated site.

In head and neck treated group, T1 percentage was decreased from 56.5% to 54.9%, T4 percentage was decreased from 36.4% to 34.1% but T8 percentage was increased from 23.5% to 24.9% resulting in decrement of T4/T8 ratio from 1.57 to 1.39. In thorax treated group, T1 percentage was decreased from 56.5% to 55.5%, T4 percentage was decreased from 36.8% to 33.6%, T8 percentage was increased from 24.7% to 25.6%, as a result, T4/T8 ratio was decreased from 1.50 to 1.34. In pelvis treated group, T1 percentage was decreased from 56.6% to 55.7%. T4 percentage was

decreased from 36.2% to 34.5%, T8 percentage was increased from 22.5% to 25.6% and T4/T8 ratio was decreased from 1.51 to 1.38. T4 percentage was decreased in all patients but the change of T8 was not uniform. In 32 of 40 patients, T8 percentage was increased, but in 8 patients, the percentage was decreased (Table 3, 4, 5). The declination in T4/T8 was not different between postoperative adjuvant treatment group and radical treatment group. T1 percentage was changed from 56.7% to 55.7% in postoperative adjuvant treatment group and from 56.4% to 55.2% in radical treatment group. T4 percentage change was from 36.3% to 34.0% in radical treatment group and was from 36.6% to 34.3% in adjuvant treatment group. T8 percentage change was from 23.2% to 24.7% in radical treatment group, and was from 24.0% to 26.2% in adjuvant treatment group. As a result, T4/T8 ratio was decreased from 1.58 to 1.4 in radical treatment group and decreased from 1.57 to 1.33 in adjuvant treatment group (Table 6, 7). Statistically, T1 change in head and neck treated group, T1 and T8 change in thorax treated group were not significant.

Table 4. T lymphocyte Change in Thorax Treated Group (n=14)

	Before RT	Immed. after RT	t-value	significance
T1(%)	56.5±1.94	55.5±1.43	2.05	NS
T4(%)	36.8±1.93	33.6±1.96	4.30	$p<0.01$
T8(%)	24.7±2.68	25.6±3.74	1.20	NS
T4/T8	1.50±0.17	1.34±0.22	4.73	$p<0.01$

Table 5. T Lymphocyte Change in Pelvis Treated Group (n=14)

	Before RT	Immed. after RT	t-value	significance
T1(%)	56.6±1.73	55.7±2.18	2.72	$p<0.05$
T4(%)	36.2±1.73	34.5±1.08	3.08	$p<0.01$
T8(%)	22.5±2.25	25.6±3.86	4.39	$p<0.01$
T4/T8	1.51±0.78	1.38±0.20	8.42	$p<0.01$

Table 6. T lymphocyte Change in Radical Treatment group (n=22)

	Before RT	Immed. after RT	t-value	significance
T1(%)	56.4±1.65	55.2±1.71	4.04	$p<0.01$
T4(%)	36.3±1.52	34.0±1.46	5.22	$p<0.01$
T8(%)	23.2±2.56	24.7±3.36	2.47	$p<0.05$
T4/T8	1.58±0.21	1.40±0.23	6.45	$p<0.01$

Table 7. T lymphocyte Change in Postoperative RT Group (n=18)

	Before RT	Immed. after RT	t-value	significance
T1(%)	56.7±2.10	55.7±1.78	3.90	$p<0.01$
T4(%)	36.6±1.98	34.3±1.50	3.97	$p<0.01$
T8(%)	24.0±3.21	26.2±1.21	3.37	$p<0.01$
T4/T8	1.57±0.21	1.33±0.18	6.36	$p<0.01$

DISCUSSION

With the specific monoclonal antibodies that define the helper T cell and inducer T cell populations in man, we studied the influence of radiation therapy on the helper/suppressor ratio in the peripheral blood of patients. There are conflicting data concerning the differential effects of radiation therapy on T lymphocytes and on T lymphocyte subpopulation determined by Fc-receptors for IgG and IgM. There is little correlation between T cell subsets defined by Fc-receptors and those defined by monoclonal antibodies[6,19~23]. Radiation therapy is associated with lymphocytopenia[4,24~31] and depressed T cell function as assessed by standard in vitro lymphoproliferative assays with mitogens and allogenic cells[25~29,31,32,34]. The deleterious effects of radiation therapy seems to relate directly to the volume of blood, lymph nodes, or bone marrow within the irradiated volume, and changes have been observed after radiation therapy to the head and neck region, mediastinum, pelvis, and skull[4,25~32].

Some authors claimed that lymphocyte-immune deficiency induced by radiotherapy led to decrease in the survival rate for the group treated by postoperative radiotherapy[10,12]. But such assumptions are refuted by many authors after a careful reanalysis of the clinical data[7~9]. Moreover, the theoretical basis for the concept of a detrimental effect are questionable[11,33]. There are conflicting reports whether the helper/suppressor ratio is increased or decreased after radiotherapy. Many in vitro functional study indicate that suppressor T cells are relatively radiation-sensitive[5,34~36]. But the numbers of lymphocytes found in the peripheral blood under normal conditions is dependent upon a number of factors such as repair capacity, cell kinetics, immune status of patients. Several studies showed contradictory result.

Some studies showed decrement in helper/sup-

pressor ratio after radiation therapy[6,15]. Job suggested that the decrease of the helper/suppressor ratio is general effect of radiation therapy and can be observed also under local therapy[6]. On the other hand, report by Schulof et al showed increased helper/suppressor ratio which studied the T cell subset change after mediastinal irradiation for lung cancer only[3].

Our data showed declination of helper/suppressor ratio after radiotherapy in almost all patients except 4 patients who showed increment. Three of which were thorax treated patients. Schulof commented that it is possible that radiotherapy administered to peripheral lymphoid tissues leads to changes in T cell subset percentage different from those observed following primary mediastinal irradiation[3]. It may be presumed that T cell subpopultion change in mediastinal irradiation group can be different from other site treatment group because of involvement of thymus in the treatment fields. As well known, T cell derived from pluripotent hematopoietic stem cells are maturated by the acquistition of cell surface determinants including antigen-specific receptors and this occurs in thymus. Of the various lymphoid tissues, the response of the thymus to radiation injury appears to be the most complex and is least well understood[37]. In our study the degree of change of helper/suppressor ratio depends neither on the irradiated volume nor on the radiation dose delivered, that is, there was no difference in T cell percentage change and helper/suppressor ratio change among head and neck treated group, thorax treated group and pelvis treated group. Although the delivered radiation dose was 1,000~2,500 cGy higher in the radical treatment group than in the adjuvant treatment group, the helper/suppressor ratio change was not different each other. Job et al studied the influence of radiation therapy on lymphcyte subpopulations in 17 patients undergoing aduvant radiation therapy for primary breast cancer, and eight patients receiving brachtherapy and external beam irradiation for primary cancer of uterus. Similar effect of radiation therapy was observed in the postoperative breast cancer group and in the uterus cancer group. Numerically, the changes were more pronounced in the uterine cancer group who received higher doses of radiotherapy. The changes in the helper/suppressor ratio were not as pronounced as in the patients receiving total nodal irradiation. They postulated that degree of changes seems to be dependent on the number of lymph nodes in the radiation field and/or the dose applied to lymphoid organ[6]. The percentage of T and B lymphocytes are known to remain constant before and after radiotherapy due to non-selective depression[6,38]. As well known, radiation therapy also induces intrinsic T cell functional deficincies. T cell function can be assayed by proliferative response to phytohemagglutinin (PHA) or by allogenic mixed lymphocyte response (MLR) to stimulator cells such as mitomycin C-treated pooled allogenic mononuclear cells[3,39]. The mechanism by which radiation therapy induces a depletion of circulating T cell and impairs intrinsic T cell functions are unknown. Several papers were published about the roles of biologic reponse modifier in minimizing and restoring radiation-induced immune derangement such as interleukin-2, levamisole, thymosin alpha-1[3,4,40]. But the beneficial effects of such modifiers are still not conclusive.

Moreover, it is not well known about the role of human T cell subsets in the immunobiological relationship between tumor and host in vivo. Therefore distinctive conclusion can not be drawn as to an effect of radiotherapy on the immune system in general (stimulatory vs suppressive), or in the specific situation of immune defence mechanisms against cancer[6]. Recently, Pillai et al reported an result of analysis of lymphocyte subpopulations done in the patients with uterine cervical cancer before and at differential intervals after the end of radiotherapy. Distinctive patterns of lymphocyte subset distribution were seen in a comparison between patients who were disease free and those with recurrent disease. The helper cell counts and helper/suppressor ratio differed between the two groups, with the consistent lower values during the follow-up in recurrent group[4]. Although the clinical significance of T cell subset change remains still to be determined, it may be thought that the prognosis of patient may be related to the capability to control the immunological change caused by insult such as radiation therapy rather than the direct effects of radiation on immune system. Further long term follow-up of T cell subset change in study population of our paper together with more collected follow-up data may present another informative data.

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= 국문초록 =

단클론항체를 이용한 방사선치료 직후의 T 임파구아형의 변화에 관한 연구

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1991년 6월부터 1991년 12월까지 경상대학교병원 치료방사선과에서 방사선치료를 시행받은 40명의 환자에 대하여 치료직전 및 치료종료직후에 있어서의 T 임파구의 비율 및 T임파구 아형의 구성비의 차이를 비교분석하였다. 연구대상이 된 40명의 환자중 12명은 두경부에, 14명은 흉부에, 14명은 골반부에 방사선치료가 시행되었으며, 22명은 방사선치료만을, 18명은 수술후 방사선치료를 시행받은 환자들이었다. 전환자에 대한 방사선치료직후의 T임파구 및 그 아형의 구성비율의 변화를 살펴보면 총 T임파구의 비율은 56.5%에서 55.4%로 감소하였고, 조력 T임파구의 비율은 36.5%에서 34.1%로 감소한 반면 억제 T임파구의 비율은 23.6%에서 25.4%로 증가하여 조력 T임파구와 억제 T임파구의 비는 1.53에서 1.37로 감소되는 결과를 나타내었다.

치료부위에 따른 변화양상의 차이는 거의 없었고, 흉부에 방사선치료를 시행받은 3명과 두경부에 치료를 받은 1명에 있어선 방사선치료 직후의 조력 T임파구와 억제 T임파구의 비가 치료전에 비하여 오히려 증가되어 있었다. 또한 수술후 방사선치료를 시행받은 군과 방사선치료만을 시행받은 군사이에서 조력 T임파구와 억제 T임파구비의 변화의 차이는 관찰할 수 없었다.

본 연구로부터 방사선치료직후, 총 T임파구 및 조력 T임파구의 비율이 감소함을 확인하였고 조력 T임파구와 억제 T임파구의 비는 감소하되 감소의 크기는 치료조사야의 크기에 영향을 받지 않고, 또한 방사선량의 차이에 의해서 조력 T임파구의 비율이 더욱 현저하게 감소하는 것은 아니라는 것을 관찰할 수 있었으나 이는 좀 더 많은 수의 환자와 장기간의 추적 연구를 통해 확증되어야 할 것이며, 장기간에 걸친 변화양상과 환자의 예후와의 상관관계를 밝히기 위한 계속적인 추적연구가 있어야 할 것이라는 결론을 얻었다